Virus Structure & Assembly

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The Role of Lipid Rafts in Virus Assembly. Identification and Characterization of Microdomain Partitioning Factors of the HIV-1 Glycoprotein gp41 using Flim-FRET and Fluorescence Anisotropy Microscopy

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Recent experimental results indicate that host cell invasion as well as assembly and budding of the Human Immunodeficiency Virus (HIV) are highly cholesterol dependent. Supposably, cholesterol enriched plasma membrane microdomains, so called rafts, play an important role in different steps of the virus lifecycle. However, the exact function and molecular background of this sensitivity to bilayer composition remains unknown.

We produced different variants of the HIV transmembrane protein gp41 labelled with a yellow fluorescent protein. Fluorescence lifetime imaging microscopy was used to report Förster Resonance Energy Transfer (FRET) between a raft marker labelled with a cyan fluorescent protein and gp41 chimeras in living cells. Since it is highly distance dependent, occurring FRET reflects a co-clustering of both fluorescent protein species in microdomains. By comparison of FRET efficiencies from different truncation and mutation variants of gp41, the Cholesterol Recognition Amino Acid Consensus (CRAC) was identified as main determinant of the protein's raft partitioning. Whereas localization and trafficking of the fusion proteins resembled reported wildtype behaviour, FACS experiments revealed a remarkable influence of CRAC mutations on plasma membrane perturbation properties of gp41. Furthermore, using fluorescence polarization anisotropy microscopy it could be shown, that wildtype gp41 oligomerization occurs at the plasma membrane. Oligomerization of CRAC mutants was found to be significantly impaired. This suggests a pooling function of lipid rafts not only for interactions with other viral components but also for assemblies of functional homo-oligomers.

This study is to our knowledge the first live cell approach characterizing gp41 raft partitioning factors and relating lateral plasma membrane sorting to distinct protein functions and properties.The reported raft dependent oligomerization might be representative for general mechanisms of microdomain-facilitated protein interactions.

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Intracellular Dynamics of HIV-Gag: The Role of Calcium and the Activation of Phospholipase C

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The assembly of the human immunodeficiency virus type 1 occurs at the plasma membrane or within late endosomes/multivesicular bodies, and is determined by the polyprotein Gag. This structural protein is also the only viral component necessary and sufficient for the assembly and the release of virus-like particles (VLPs). It is well known that participation of host cell components is particularly required for any of the many Gag-encoded functions. In particular, as already shown by L.S. Ehrlich et al. 2010, the activation of the phospholipase C and the inositol-(1,4,5)-triphosphate receptor, resulting in an increase of intracellular calcium concentration, are both required for efficient Gag trafficking and VLPs release.

So far, it is still unclear whether the Gag protein itself or a cellular factor specifically activates this signaling pathway for the virus release process.

We are interested to investigate the activation and the role of the PLC signaling pathway in Gag-expressing cells, and how the intracellular calcium concentration can influence the dynamics of Gag and the VLPs release-process.

HeLa cells transfected with Gag fused with a fluorescent protein (e.g. EGFP, EYFP) are our principal tool to investigate the intracellular localization of the viral protein upon activation or inhibition of the PLC signaling pathway. Acetoxymethylester-derivates of fluorescent indicators are used to study variations of the calcium concentration in live cells. Chemical inhibitors and RNAi technology are utilized to turn off the PLC pathway at different steps and time points. Total Internal Reflection Fluorescence microscopy, Förster Resonance Energy Transfer methods and co-immunoprecipitation are applied to study the localization of Gag at the plasma membrane or in the cell-interior, and to identify interactions with specific binding partners of the host cell.

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The HIV-1 Pre-Integration Complex: Structural and Functional Role of INI1 and LEDGF, Cellular Cofactors of Viral Integrase

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Virally encoded integrase (IN) proteins perform several important steps in the life cycle of retroviruses. During the early events of viral replication the RNA genome is converted into its cDNA copy which upon interaction with cellular and viral proteins generates the pre-integration complex (PIC). IN is a permanent component of the PIC. The Integrase Interactor Protein 1 (INI1) a homolog of yeast SNF5 and the lens epithelial derived growth factor (LEDGF) have been shown to interact with HIV-1 IN. In order to understand the mechanisms of the INI1 - mediated inhibition and/or activation functions in the early stage of HIV-1 infection, we analyzed the structure-function relationships of a quaternary complex comprising the full length wild type HIV-1 IN, the full length wild-type LEDGF, the INI1 IN binding domain (173-290) and viral U5 DNA. The stoichiometry of the components is 4/2/2/2, as shown by mass spectrometry and FCS. We determined for the first time the binding constants of U5 vDNA for IN by fluorescence anisotropy and found that the dissociation constants of IN/LEDGF and IN/LEDGF/INI1 for U5 vDNA remained in the same order of magnitude, while INI1 when bound to the IN/LEDGF complex inhibited the 3'processing reaction. CryoEM and in vitro functional analysis show that INI1, located within the cellular DNA binding site, inhibits the 3' processing but not specific viral DNA binding. INI1 stabilizes the highly flexible integrase in a nonproductive conformation. Taken together, our data suggest that the role of INI1 could be to stabilize the highly flexible IN protein in a conformation that prevent non-specific interaction and auto integration during nucleosome targeting. Our results provide the basis for a novel type of integrase inhibitors (conformational inhibitors).

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Translating Bulk Measures of Capsid Assembly Progress into Insights on Fine-Scale Kinetics and Pathways

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Virus capsid assembly has become a key model system for studies of complicated self-assembly processes, attracting considerable interest from the biophysical modeling community. Simulation methods have proven valuable for gaining insight into the space of possible kinetics and mechanisms of capsid assembly, but they have so far been able to say little about the assembly kinetics of any specific virus. It is not currently possible to directly measure the detailed interaction rates needed to parameterize a model and there is only a limited amount of experimental evidence of assembly kinetics available to constrain possible pathways, almost all of it gathered from *in vitro* studies of purified coat proteins. We have developed methods to address this problem that use data fitting algorithms to learn rate parameters consistent with both structure-based rule sets and experimental light scattering data on bulk assembly progress *in vitro*. Our method combines ideas from gradient-based and response-surface local optimization methods with a heuristic global search strategy to find parameter fits that can approximately reproduce experimental measures of assembly progress. We have applied these methods to data from three capsid systems - human papillomavirus (HPV), hepatitis B virus (HBV), and cowpea chlorotic mottle virus (CCMV) - with the resulting fits suggesting three very different assembly mechanisms. Work is continuing to refine the learned rate parameters and pathways and explore how these mechanisms might change when computationally translated into more realistic representations of the assembly environment *in vivo* in order to more accurately model the assembly of viral capsids in living cells.

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Uncoating of Mature HIV Capsids Driven by Reverse Transcription

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Until recently it was a common notion that reverse transcription (RTion) in retroviruses, including HIV, takes place within the cytoplasm of the infected cell after uncoating of the mature capsid. However, accumulating evidence suggests that at least some RTion happens inside the capsid, and may be driving the uncoating. In this theoretical study we consider the problem of mature HIV capsid uncoating driven by polymerization of double stranded (ds) viral DNA by the